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LABORATORY INVESTIGATION MYOCARDIAL INFARCTION

Survival after an experimental myocardial infarction: beneficial effects of long-term therapy with captopril

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ABSTRACT Although vasodilator therapy has been shown to improve functional capacity in patients with congestive heart failure, there is no evidence that such therapy can prolong survival. Coronary artery ligation in the rat was used to produce a wide range of myocardial infarct sizes and a resultant spectrum of left ventricular dysfunction. To determine the relationship between size of myocardial infarction and long-term survival and to test the hypothesis that long-term therapy with captopril could improve survival after myocardial infarction, 302 rats were randomly assigned to either placebo or captopril therapy 14 days after coronary artery ligation. The animals were kept in a laminar flow unit and followed daily for a 1 year period or until spontaneous death. Size of myocardial infarction was determined by planimetry of serial histologic sections of the left ventricle. One year survival in placebo-treated rats decreased markedly in direct relation to increasing size of infarction (from 71% in noninfarcted rats to only 8% in rats with large infarcts). Long-term captopril therapy prolonged the survival of rats with infarcts ($p < .02$). The most marked improvement in survival was noted in the animals with infarcts of moderate size, in which 1 year survival was 21% in the placebo-treated rats and 48% in the captopril-treated rats. Thus, in this experimental preparation of myocardial infarction and left ventricular dysfunction, survival was inversely related to size of infarction. Long-term therapy with captopril, which we had previously shown to improve left ventricular function and lessen dilatation in the chronic phase of infarction, also had a pronounced effect on prolonging survival in this preparation of chronic infarction.

Circulation 72, No. 2, 406-412, 1985

THE DIAGNOSIS of congestive heart failure has a grave prognosis.^{1,2} In the Framingham Study, the 5 year mortality rate was greater than 50% for individuals newly diagnosed as having congestive heart failure.¹ In more recent studies, the median survival of patients with less clearly defined durations of ventricular dysfunction was often measured in months, not years.^{3,4} Although congestive heart failure is a syndrome that encompasses multiple etiologies, the severity of the cardiac dysfunction appears to have greater prognostic significance than does the specific cause of disease.⁴ The use of vasodilators, especially the angiotensin-converting enzyme inhibitors, in the treatment

of patients with severe heart failure has led to an improvement in functional capacity.⁶⁻¹¹ However, there is no evidence that these therapeutic interventions result in a prolongation of life in individuals with rather advanced congestive heart failure.¹²⁻¹⁴

Coronary artery ligation in the rat provides an animal preparation of myocardial infarction in which a wide range of infarct sizes and left ventricular dysfunction can be produced.^{15,16} Since we have shown that captopril improves ventricular performance in this preparation of heart failure,¹⁷ the present study was designed (1) to determine the relationship between size of infarction and long-term survival, and (2) to test the hypothesis that a therapeutic agent that has a beneficial effect on ventricular performance can prolong survival after experimental myocardial infarction.

Methods

Study Design. Commercially obtained virgin female Wistar rats (Wistar, Jersey Biological Supply), ranging in age from 16 to 20 weeks, were housed in our animal facility for at least 2 weeks before operation. Left coronary artery ligation or a sham operation was performed in each by a previously described method.^{15,16} Since this study was concerned with the chronic phase

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Received March 5, 1985; revision accepted May 2, 1985.

Dr. J. Pfeffer is the recipient of a Research Career Development Award HL 01186 from the NHLBI of the National Institutes of Health. Dr. M. Pfeffer is the recipient of an Established Investigatorship of the American Heart Association.

of myocardial survival after myocardial infarction. The balance of the study was to determine the effect of captopril on survival after myocardial infarction. The study was conducted in a laminar flow unit. The study was conducted in a laminar flow unit. The study was conducted in a laminar flow unit.

Animals were assigned to either placebo or captopril therapy. The study was conducted in a laminar flow unit. The study was conducted in a laminar flow unit. The study was conducted in a laminar flow unit.

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of myocardial infarction. 14 days after the operative procedure, all survivors (302) were briefly anesthetized with ether and electrocardiograms were obtained with a previously described nine-lead system.¹⁸ At the time of anesthesia a metal identification tag was affixed to the nape of the neck of each rat. A balanced-block randomization process was used to allocate animals to either placebo or captopril therapy groups. This randomization process was stratified according to three arbitrary electrocardiographic groups. The first group had a normal QRS complex without evidence of a pathologic Q wave in limb leads I or aVL; the second group had a Q wave (≥ 1 mV) in either of leads I or aVL, yet maintained a precordial R wave total of 10 mV or greater; and the third group had both abnormal limb-lead Q waves and a sum of precordial R waves that was 10 mV or less. This electrocardiographic classification system was used only in an attempt to maintain a balance of rats with and without infarction in the placebo and captopril groups, and not for the subsequent survival analysis, which was performed with the histologic determinations of the size of myocardial infarction only.

Animals were housed in clear polyethylene cages (up to a maximum of four rats per cage) and placed according to therapy assignment and time of entry into the study. All animals were allowed free access to food and the assigned therapy (water or captopril, 2 g/liter of drinking water). The room was temperature and humidity controlled and had a 12-hr light-dark cycle. The cages were maintained on two racks, each of which abutted one side of a double-sided laminar flow unit (Germ-Free Laboratories). Cages with animals assigned to placebo or captopril were alternately placed on the rack. An additional 39 rats in which no operation was performed were selected from the same shipments used for this study and maintained concurrently with the study animals. Each animal was followed until death or for up to 1 year after the operative procedure.

Cages were inspected for dead animals twice daily on weekdays and daily on weekends and holidays. The date of each death was noted and the carcass was weighed and a gross post-mortem examination was performed. The thoracic contents were removed en bloc and placed in formalin along with the metal identification tag. This identification number, which in itself did not discriminate between the therapy groups, was used in all subsequent pathologic processing. All survivors at 1 year after infarction were anesthetized with ether and the heart of each was arrested with intravenous KCl. The same coded pathologic evaluation was performed on these animals as was conducted on all spontaneous deaths.

Pathology. The formalin-fixed lungs were dissected from the heart and examined for areas of consolidation. The following scoring system for thoracic infections was applied to each specimen: grade 1, no gross infection; grade 2, small area(s) of consolidation involving less than one-third of the estimated lung volume; and grade 3, large area(s) of consolidation involving more than one-third of the estimated lung volume or diffuse thoracic infection without localization. The heart was examined and the aorta and great vessels were trimmed from the ventricles. The right ventricular free wall was dissected from the left ventricle and both ventricles were weighed separately. The left ventricle was dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Transverse 50 μ m sections were cut from the apex to the base of the left ventricle, every twentieth section of which (representing 1 mm intervals) was mounted and stained with Masson's trichrome from which hematoxylin was omitted to provide a clearer discrimination between fibrous scar and muscle.

A slide of each section then was projected onto a screen for the planimetric measurement (Numonics Corp.) of size of infarction. The endocardial and epicardial circumferences as well

as the lengths of scar for each of these surfaces were measured for each section. The lengths of scar and of circumference were summed separately for each of the epicardial and endocardial surfaces and the sums were expressed as a ratio of scar length to ventricular circumference for each surface. These two ratios were then averaged and expressed as a percentage to define size of infarction. Since serial sections of the entire left ventricle were evaluated, the size of infarction determined represents the percent of the left ventricular endocardial and epicardial surface areas occupied by fibrous scar tissue.

Statistical analysis. The following four previously determined classifications of size of infarction were used in the analysis: noninfarcted, 4.99% or less; small, 5% to 19.99%; moderate, 20% to 39.99%; and large, 40% or more of the left ventricular surface area occupied by fibrous scar tissue. A one-way analysis of variance was used to determine whether the randomization process resulted in groups that were matched for age and body weight at the time of operation. The t test was used to test for matched treatment (water to captopril) groups within each of the four categories of size of infarction with respect to these variables. Log-rank tests were conducted to determine whether size of infarction altered 1 year survival in the untreated group. In these untreated rats, the effect of size of infarction also was analyzed as a continuous variable using the Cox model to calculate the relative risk of 1 year mortality with increasing infarct size.

Comparisons of survival in untreated and captopril-treated rats were also performed separately by log-rank tests for all of the rats with and without infarction. Similar analyses were performed for each of the three groups based on size of infarction. Since potential differences in rates of thoracic infections could have contributed to a possible alteration in group survival, results in animals with a thoracic infection score of grade 3 were treated as censored observations and the log-rank tests for comparisons between treatment groups were repeated as a separate analysis. An additional log-rank analysis of 1 year survival in the rats without infarction that either did or did not undergo operation was also conducted.

Results

Seven (three untreated and four captopril-treated) of the 302 animals entered into the study were excluded. One rat died during the brief interval between randomization and placement into the therapy cage, and in six other animals the determination of size of infarction was not possible because of postmortem cannibalization.

The randomization process produced a balance between the therapy groups with respect to both the sample size and the distribution of infarct sizes in each classification (table 1). At the time of operation there were no significant differences in age between the therapy groups for any of the infarct size classifications or among the untreated rats with infarcts of different sizes. However, among the groups of captopril-treated rats with infarction there was a significant difference in age (of about 1 week) that was of questionable biologic significance. There were no significant differences in body weight among the groups for any of the infarct size classifications (table 1).

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TABLE 1

Physical characteristics and distribution of rats with and without infarcts in the various groups

	No infarct	Infarct		
		Small	Moderate	Large
Sample size (No.)				
H ₂ O	62	15	29	40
CAP	63	13	35	37
Infarct size (%)				
H ₂ O	0	12.7 ± 1.3	33.4 ± 0.8	47.3 ± 0.7
CAP	0	12.7 ± 1.4	33.1 ± 0.9	46.4 ± 0.7
Age (weeks)				
H ₂ O	20.5 ± 0.3	20.6 ± 0.6	20.0 ± 0.4	19.6 ± 0.2
CAP	20.7 ± 0.3	20.8 ± 0.6	20.0 ± 0.4	19.4 ± 0.2
Body weight (g)				
H ₂ O	295 ± 4	298 ± 5	294 ± 5	304 ± 3
CAP	295 ± 4	282 ± 6	296 ± 4	304 ± 3

Results are expressed as mean ± SEM.

H₂O = untreated rats; CAP = captopril-treated rats.**p* < .05 compared with "no infarct" group.

Within either treatment group the 1 year mortality for the sham-operated rats was not significantly different than that for rats that did not undergo operation. The "all causes," 1 year mortality for the rats assigned to water only was increased (*p* < .0001) as a function of size of infarction (figure 1). The median survival for the animals without infarction was greater than the 365 day observation period and was therefore undefined. Animals with small, moderate, and large infarctions demonstrated reduced median survival durations of 360, 228, and 146 days, respectively. In these animals, the relative risk of death increased progressively with increasing size of infarction (figure 2). Untreated rats with infarcts of between 20% and 29.9% formed the smallest infarct size group to have a relative risk that was significantly greater (*p* < .01) than that in rats without infarction. The risk of death increased steeply

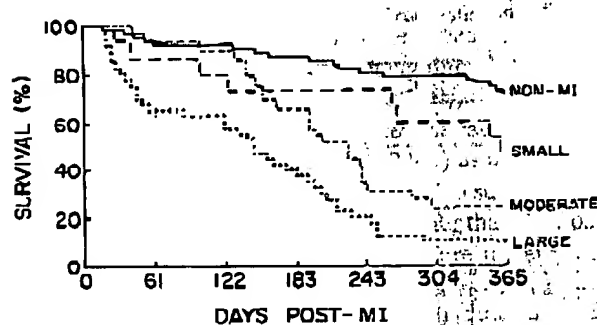


FIGURE 1. Survival of untreated animals without infarction and of those in the three predetermined infarct size groups (small, 5% to 19.9%; moderate, 20% to 39.9%; and large, ≥40% of left ventricular surface).

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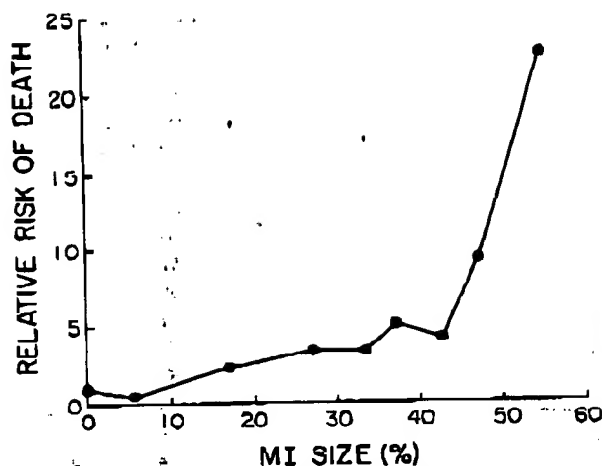


FIGURE 2. Risk of death for untreated rats with infarction relative to operated rats without infarction (infarct size, 0%). The median value of eight categories of size of infarction was used to define the relative risk of death for animals with infarcts.

as infarct size increased and became 22.6 times greater in untreated rats with extensive (≥50%) infarctions than in those without infarction.

In the rats without infarction receiving captopril, survival was quite similar to that in the untreated rats without infarction (figure 3, A). In contrast, all captopril-treated rats with infarctions demonstrated a reduced 1 year mortality rate (*p* = .01) compared with untreated rats with infarction (figure 3, B). The median survival for the entire group of rats with infarction was 197 days for the untreated animals and 260 days for the captopril-treated group. This improved survival of the captopril-treated rats was observed uniformly across all infarct size groups, although the difference was not always statistically significant within each subgroup (figure 4). The overall 1 year mortality for rats with small (5% to 19.9%) infarctions did not differ (*p* = .23) in treated and untreated groups (figure 4, A). In contrast, rats with moderate (20% to 39.9%) infarctions treated with captopril exhibited a marked improvement (*p* = .02) in 1 year survival compared with untreated rats (figure 4, B). Although there was an initial trend toward improved survival in rats with large (≥40%) infarctions receiving captopril, a long-term beneficial effect was not sustained (*p* = .24) (figure 4, C).

Censoring of results in rats with thoracic infections was performed to reduce the confounding influence of a potential noncardiac cause of mortality. The incidence of grade 3 infections (an infection likely to account for death) did not differ between treatments or infarct size groups. Indeed, censoring for high-grade

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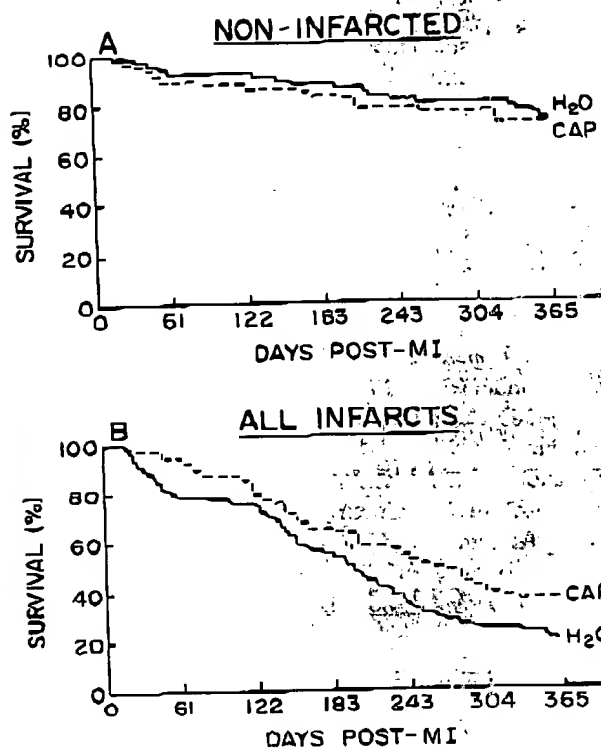


FIGURE 3. A. Survival of untreated (H₂O) and captopril-treated (CAP) animals that underwent operation but did not sustain myocardial infarction. No differences were observed between therapy groups. The median survival for both groups was undefined since it was greater than the 365 day observation period. B. Survival of all untreated (H₂O) and captopril-treated (CAP) animals that sustained myocardial infarction. Survival for infarcted animals receiving captopril was prolonged compared with that in untreated rats ($p < .02$). The median survival for the untreated rats was 187 days and that in CAP animals was 260 days.

thoracic infections actually reinforced the relationship between increasing size of infarction and reduced mortality and the beneficial effects of captopril therapy in rats with chronic myocardial infarctions. With the use of this censored analysis the median survival for rats without infarcts and those with small infarcts in both therapy groups was undefined (greater than the 365 day observation period). In rats with moderate infarcts the median survival was 228 days for the untreated rats and greater than 365 for the captopril-treated rats. The median survival for rats with large infarcts was unchanged by therapy (untreated 184 days and captopril-treated 205 days).

Discussion

Therapy for heart failure has focused on the relief of symptoms of congestion by the restriction of physical activity and dietary sodium intake and the use of phar-

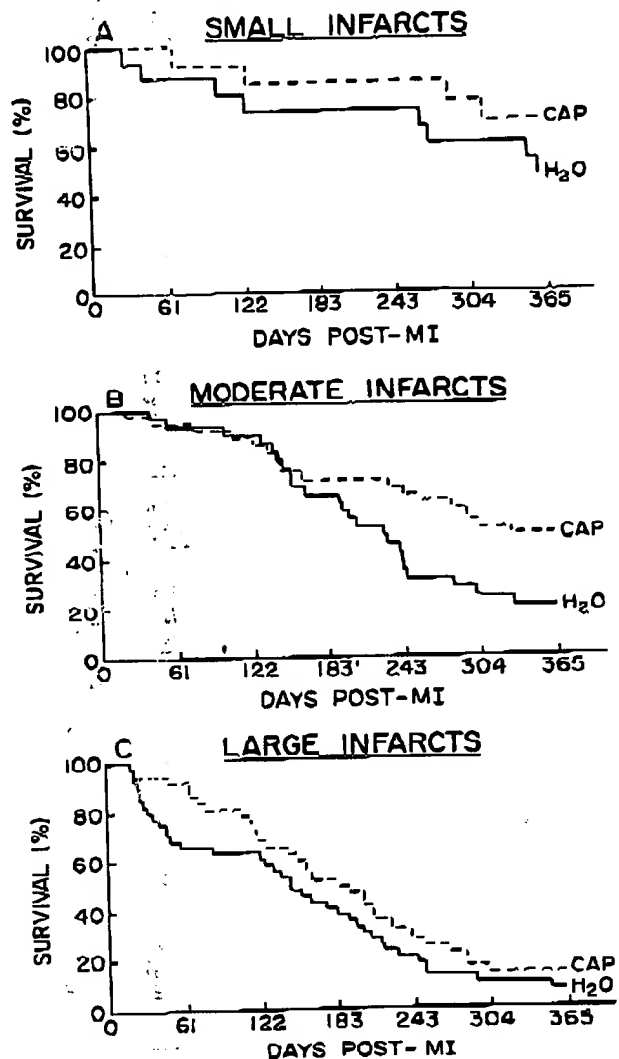


FIGURE 4. Survival of untreated (H₂O) and captopril-treated (CAP) rats with small (A), moderate (B), or large (C) infarctions. In the small infarct groups the median survival was 360 days and that in the untreated and CAP groups was undefined (greater than the 365 day observation period). In the moderate infarct group the median survival was 228 and 329 days for the untreated and CAP rats, respectively. In the large infarct groups the median survival was 146 and 181 days for the untreated and captopril-treated rats, respectively.

macologic agents such as diuretics. Cardiac glycosides are also widely used for the treatment of heart failure, although their efficacy in patients with sinus rhythm has been questioned. Vasodilating drugs recently have been introduced as adjunctive therapy in patients with congestive heart failure because of the hemodynamic improvements observed during their short-term administration. Although long-term beneficial hemodynamic responses to vasodilators have not been observed uni-

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coronary artery ligation. By this time the acute inflammatory process of infarction is over and scar formation is complete.²⁴ None of the deaths within the observation period was associated with hemothorax, an indicator of myocardial rupture.

An excellent balance of infarct sizes between the untreated and captopril-treated groups was achieved by the randomization process. The survival of the rats without infarction from both treatment groups was identical (figure 3, A). When all infarcted animals were pooled separately for each treatment, the survival of the captopril-treated group was superior to that of the untreated cohort (figure 3, B). Subclassification according to size of infarction revealed that this prolongation of survival was not distributed uniformly across all infarct size groups. The rats with small infarcts made up the smallest number of animals with infarction and a log-rank test did not reveal a difference in survival between the treatment groups. In contrast, in the rats classified as having moderate infarctions there was a prolongation of survival in the animals allocated to the captopril group (figure 4, B). Of note is the fact that this difference in survival was observed primarily in the late period after the experimental infarction: the captopril-treated animals did not demonstrate the late mortality that was observed in the untreated group. In the rats with large myocardial infarctions there was no overall difference in survival during the 1 year observation period and, although there appeared to be an early prolongation of survival in the captopril-treated animals, this trend was not sustained over the predetermined observation period.

This study was designed with total mortality as the single end point; however, a second analysis was performed in which results in animals with overt thoracic infections were censored. The elimination of these data from the analysis only served to strengthen all of the above conclusions.

This study demonstrates that survival is related to size of myocardial infarction and that long-term captopril therapy can prolong the survival of animals with healed myocardial infarction and left ventricular dysfunction. The most benefit from drug therapy with respect to survival was noted in animals with infarctions of moderate size. This is in agreement with results of our prior study of the effects of long-term captopril therapy on ventricular performance and ejection size, in which rats with extensive infarctions demonstrated minimal hemodynamic benefit from long-term therapy. The implication of these results is that in severe heart failure it may be unrealistic to anticipate that captopril therapy will prolong survival. However,

this animal study underscores the need for a clinical evaluation of captopril in patients with less severe ventricular dysfunction. Indeed, an important implication of this study is that captopril therapy may be effective in prolonging life if instituted early in the course of left ventricular dysfunction after myocardial infarction and possibly in patients with other forms of early congestive heart failure.

We thank Professor Eugene Braunwald for his thoughtful review of the manuscript. The statistical consultation provided by Dr. James Anderson and Ms. Harriet F. Peterson, M.S., of the Harvard School of Public Health is gratefully acknowledged. Data organization and analysis was assisted by the CLINFO data management and analysis system at Brigham and Women's Hospital, Boston, supported by GCRC No. 5 M01 RR00888-10 CLR. We thank Mrs. Angela Perry for her secretarial assistance in the preparation of this manuscript. Captopril was generously supplied by Drs. John C. Alexander and Zola P. Horowitz of the Squibb Institute for Medical Research.

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